Research in Fisheries Contaminants and Toxicology Conducted by the Contaminants Research Unit, Fisheries and Environmental Sciences Division, Halifax Component, in 1983

J. F. Uthe, Editor

Halifax Fisheries Research Laboratory
Halifax, N. S., B3J 2S7

April 1984

Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 1757

Fisheries and Oceans / Pêches et Océans

Canada

2008-05991/

Canadian Manuscript Report of Fisheries and Aquatic Sciences

These reports contain scientific and technical information that represents an important contribution to existing knowledge but which for some reason may not be appropriate for primary scientific (i.e. *Journal*) publication. They differ from Technical Reports in terms of subject scope and potential audience: Manuscript Reports deal primarily with national or regional problems and distribution is generally restricted to institutions or individuals located in particular regions of Canada. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries management, technology and development, ocean sciences, and aquatic environments relevant to Canada.

Manuscript Reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report will be abstracted by *Aquatic Sciences and Fisheries Abstracts* and will be indexed annually in the Department's index to scientific and technical publications.

Numbers 1-900 in this series were issued as Manuscript Reports (Biological Series) of the Biological Board of Canada, and subsequent to 1937 when the name of the Board was changed by Act of Parliament, as Manuscript Reports (Biological Series) of the Fisheries Research Board of Canada. Numbers 901-1425 were issued as Manuscript Reports of the Fisheries Research Board of Canada. Numbers 1426-1550 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Manuscript Reports. The current series name was changed with report number 1551.

Details on the availability of Manuscript Reports in hard copy may be obtained from the issuing establishment indicated on the front cover.

Rapport manuscrit canadien des sciences halieutiques et aquatiques

Ces rapports contiennent des renseignements scientifiques et techniques qui constituent une contribution importante aux connaissances actuelles mais qui, pour une raison ou pour une autre, ne semblent pas appropriés pour la publication dans un journal scientifique. Ils se distinguent des Rapports techniques par la portée du sujet et le lecteur visé; en effet, ils s'attachent principalement à des problèmes d'ordre national ou régional et la distribution en est généralement limitée aux organismes et aux personnes de régions particulières du Canada. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du Ministère des Pêches et des Océans, notamment gestion des pêches, techniques et développement, sciences océaniques et environnements aquatiques, au Canada.

Les Manuscrits peuvent être considérés comme des publications complètes. Le titre exact paraît au haut du résumé de chaque rapport, qui sera publié dans la revue *Aquatic Sciences and Fisheries Abstracts* et qui figurera dans l'index annuel des publications scientifiques et techniques du Ministère.

Les numéros de 1 à 900 de cette série ont été publiés à titre de manuscrits (Série biologique) de l'Office de biologie du Canada, et après le changement de la désignation de cet organisme par décret du Parlement, en 1937, ont été classés en tant que manuscrits (Série biologique) de l'Office des recherches sur les pêcheries du Canada. Les numéros allant de 901 à 1425 ont été publiés à titre de manuscrits de l'Office des recherches sur les pêcheries du Canada. Les numéros 1426 à 1550 ont été publiés à titre de Rapport manuscrits du Service des pêches et de la mer, Ministère des Pêches et de l'Environnement. Le nom de la série a été changé à partir du rapport numéro 1551.

La page couverture porte le nom de l'établissement auteur où l'on peut se procurer les rapports sous couverture cartonnée.

Canadian Manuscript Report of
Fisheries and Aquatic Sciences 1757

April 1984

RESEARCH IN FISHERIES CONTAMINANTS AND TOXICOLOGY CONDUCTED BY THE CONTAMINANTS RESEARCH UNIT, FISHERIES AND ENVIRONMENTAL SCIENCES DIVISION, HALIFAX COMPONENT, IN 1983

J.F. Uthe
Editor
Fisheries Contaminants and Toxicology Section
Fisheries and Environmental Sciences
Fisheries Research Branch
Halifax Fisheries Research Laboratory
P.O. Box 550, Halifax, Nova Scotia
B3J 2S7

(c) Minister of Supply and Services Canada 1984

Cat. No. Fs-4/1757 ISSN 0706-6473

Correct citation for this publication:

Uthe, J.F., ed. 1984. Research in fisheries contaminants and toxicology conducted by the Contaminants Research Unit, Fisheries and Environmental Sciences Division, Halifax Component, in 1983. Can. MS Rep. Fish. Aquat. Sci. 1757: iii + 12 p.

ABSTRACT

Uthe, J.F., ed. 1984. Research in fisheries contaminants and toxicology conducted by the Contaminants Research Unit, Fisheries and Environmental Sciences Division, Halifax Component, in 1983. Can. MS Rep. Fish. Aquat. Sci. 1757: iii + 12 p.

This report describes research performed in 1983 by the Halifax component of the Fisheries Contaminants and Toxicology Section of the Fisheries and Environmental Sciences Division. The research dealt with cadmium in shellfish, heavy metal interactions in lobsters fed cadmium, organochlorines in cod, interlaboratory calibrative studies, steroid hormone isolation and identification in lobsters, radioimmunoassay and the effects of acid precipitation on sexual maturation and reproduction in caged Atlantic salmon.

Keywords: cadmium, organochlorines, shellfish, finfish, acid rain, steroid hormone metabolism, monitoring

RÉSUMÉ

Uthe, J.F., ed. 1984. Research in fisheries contaminants and toxicology conducted by the Contaminants Research Unit, Fisheries and Environmental Sciences Division, Halifax Component, in 1983. Can. MS Rep. Fish. Aquat. Sci. 1757: iii + 12 p.

Ce rapport décrit les recherches menées en 1983 par la Section des polluants et toxicologie des peches, Halifax, de la Division des peches et des sciences de l'environment. Les recherches ont porté sur la présence de cadmium chez les crustacés, sur les interactions des métaux lourds chez les homards à qui on a administré du cadmium, sur les organochlorures chez la morue, sur des études de calibration entre laboratoires, sur l'isolation et l'identification des hormones stéroides chez les homards, sur le dosage radio-immunologique et les effets des précipitations acides sur la maturation sexuelle et la reproduction du saumon de l'Atlantique en bassin.

INTRODUCTION

The long-term objective of the program is to assess hazards posed by chemicals to the well-being of fish stocks and their commercial utilization. The studies include the measurement of time and geographical trends in levels of contaminants in a variety of commercial species, particularly in areas of known contaminant input. In addition, the effects of contaminants on the metabolism of steroid hormones and reproduction in selected species is assessed.

In 1983, the emphasis was on cadmium (Cd) levels in lobster from the area of Belledune Harbour, New Brunswick, in scallops from Georges Bank, and on the nature of Cd and other metals in lobster. The effect of feeding Cd on levels of Cd, zinc (Zn), copper (Cu), and silver (Ag) in digestive gland and tail muscle of juvenile lobsters was determined. Work on organic contaminants was concentrated on determining the levels of a variety of common organochlorine materials in cod liver.

Studies on steroid hormone metabolism were concentrated on isolation and identification of hormones in lobster and on the application of the technique for determining the effects of acidic precipitation on Atlantic salmon held in cages in the Westfield River, Nova Scotia.

MARINE ENVIRONMENTAL CONTAMINATION

J.F. Uthe
C.L. Chou
C.J. Musial

Marine environmental contamination studies are designed to determine the levels of a variety of chemical contaminants in commercially important and other marine species, and geographical and temporal changes in these levels. In addition, levels of contaminants in selected fishery products are investigated to ensure the continued marketability of such products originating from areas of potential or actual contamination. While the research effort has been concentrated upon toxic elements, surveys and studies of routine analytical methodologies for a number of organochlorines and polycyclic aromatic hydrocarbons are also conducted. Research during the past year has been concentrated on the distribution of Cd in lobster and scallop and the chemical nature of Cd, Zn, Cu, and Ag in lobster. In cooperation with the Disease and Nutrition Section the effect of dietary Cd, on concentrations of Cd, Zn, Cu and Ag in digestive gland and tail muscle of lobster was determined.

STUDIES ON Cd IN LOBSTERS FROM BELLEDUNE, NEW BRUNSWICK

Since 1980 Fisheries and Environmental Sciences has studied and monitored pollution of Belledune Harbour, New Brunswick. Cd levels in lobster (Homarus americanus) captured within and around the harbour in 1980 were high enough to warrant closure to the commercial fishing of lobsters and imposition of a "quarantine" fishing zone in which the lobsters that were captured underwent mandatory special processing and inspection to ensure wholesomeness of the product (Uthe and Zitko 1980; Uthe et al. 1982a, 1983). In May and June 1983 a survey of Cd in the raw digestive glands and cooked meat (tail and claw meat pooled from one animal) was carried out to determine whether: 1) the harbour is still sufficiently contaminated to continue the fishing ban; 2) the harbour could be reopened to commercial fishing as an enlarged "quarantine" fishing zone; 3) the "quarantine" fishing zone surrounding the harbour is still warranted and of the right geographical area.

Five sites, two within the harbour (Harbour West and Harbour East), two in the controlled fishing zone (L1E and L4E) and one control site (Heron Island) were sampled annually (Fig. 1). Table 1 shows the results for Cd in the digestive glands from the five sample sites and the equivalent values for 1982. Table 2 compares the results for Cd in cooked meat and digestive glands, again with the equivalent results for 1982.

Two factors confound the statistical interpretation of the results: Differences in mean animal weights are present for each sample site between 1982 and 1983. There is a significant relationship between the weight of the animal and the concentration of Cd in the digestive gland (Uthe et al. 1980) but the relationship is not tight enough to give ideal weight corrections in the analysis of covariance. In 1982, 9 of 35 animals (26%) captured at Harbour West had digestive gland Cd levels in excess of 400 mg/kg (wet wt). None of the lobsters captured at this site in 1983 had digestive gland Cd levels greater than 400 mg/kg. The lack of highly contaminated animals in 1983 is difficult to explain. Possibly such highly contaminated animals have died in the interim. Appropriate methods of statistical analysis of the data are still being developed. It may be that the drop in mean level of Cd in the digestive glands between 1982 and 1983 is due to the lack of highly contaminated animals in the 1983 catch rather than a general decrease in Cd levels within the total population. Similarly, (Table 2) there is a decrease in the level of Cd in the cooked meat between 1982 and 1983. Levels of Cd in cooked meat pools in 1983 did not exceed 1.60 mg Cd/kg (wet wt) whereas, in 1982, 6 of 21 (29%) cooked meat pools had Cd levels in excess of 1.60 mg/kg.

Statistical analysis of the data is also on-going. The decrease in cooked meat mean Cd content of animals from the Harbour west site between 1982 and 1983 was not significant, using Cochran's modified "t" test (Snedecor and Cochran 1967).

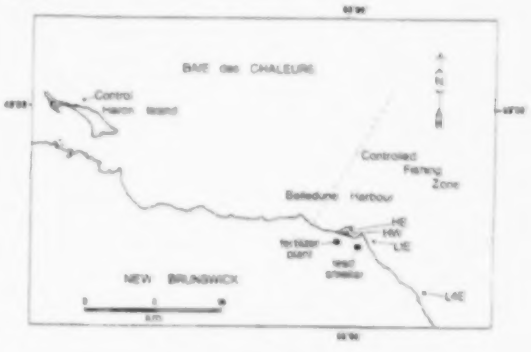

Fig 1. Lobster sampling sites at Belledune, N.B. The sites within the harbour are Harbour West (HW) and Harbour East (HE).

Table 1. Geometric mean Cd levels (mg/kg wet wt) in raw digestive glands of lobster captured within or around Belledune Harbour, New Brunswick (ranges shown in brackets).

	1982			1983		
Sample site	Mean animal wt (± S.D.)(g)	N	$\bar{X}g$	Mean animal wt (± S.D.)(g)	N	$\bar{X}g$
Heron Island (control)	355 ± 126 (137-740)	23	4.69 (2.08-10.05)	454 ± 188 (206-977)	26	4.09 (1.88-9.41)
Within Belledune Harbour						
Harbour West	503 ± 269 (158-1515)	35	147 (8.79-728)	383 ± 242 (108-1073)	40	73.1 (11.3-348)
Harbour East	542 ± 213 (286-1056)	35	76.3 (14.7-470)	436 ± 146 (153-793)	25	32.5 (8.21-254)
Within controlled fishery zone						
L1E	330 ± 153 (164-735)	36	40.4 (5.42-132)	365 ± 159 (155-686)	24	31.6 (8.39-203)
L4E	342 ± 157 (146-1037)	38	23.8 (3.66-122)	405 ± 276 (138-1124)	28	23.8 (6.53-177)

Table 2. Geometric mean Cd levels (mg/kg wet wt) in cooked meat and raw digestive gland of lobster captured within or around Belledune Harbour, New Brunswick. These animals are a subsample of those in Table 1.

Sample site	Year	Mean Animal weight(± S.D.) (g)	Cooked meat $\bar{X}g$	Cooked meat Range	Digestive gland $\bar{X}g$	Digestive gland Range	N
Heron Island (control)	1982	395 ± 114	0.03	0.02-0.03	5.32	3.51-10.1	9
	1983	410 ± 211	0.02	0.01-0.03	4.05	1.88-9.41	13
Within Belledune Harbour							
Harbour West	1982	511 ± 317	0.74	0.11-3.26	165	33.3-728	21
	1983	465 ± 252	0.47	0.11-1.59	74.2	11.3-348	25
Harbour East	1982	551 ± 209	0.23	0.04-0.76	62.5	14.7-470	12
	1983	465 ± 177	0.24	0.07-1.53	44.5	12.0-254	11
Within Controlled fishery zone							
L1E	1982	330 ± 145	0.18	0.05-0.43	47.8	10.3-134	10
	1983	297 ± 117	0.11	0.02-0.66	29.4	8.39-70.7	11
L4E	1982	406 ± 121	0.11	0.05-0.28	23.4	11.5-47.0	11
	1983	436 ± 235	0.14	0.03-1.32	20.7	9.75-157	14

Cd IN SCALLOPS

Cd is present in soft tissues of sea scallops (Placopecten magellanicus) (Ray 1980) with the highest concentrations being present in the viscera (stomach, gill, gonad, digestive gland, heart and kidney), a lower level in the mantle, and the lowest level in the adductor muscle. Adductor muscle Cd levels from scallops captured in the area of Belledune, New Brunswick ranged from 0.5-8.4 mg/kg (dry wt) and were elevated compared to levels of 0.8 mg/kg in scallops from Passamaquoddy Bay, New Brunswick, or levels of 0.07-0.20 mg/kg (wet wt) in animals from the New York Bight (Reid et al. 1982). Thus, on a wet weight basis (conversion factor 5:1), adductor muscle from Belledune area scallops could contain levels in excess of 1.0 mg Cd/kg, a level that is of concern in foodstuffs, since it is recommended that humans ingest no more than 1 µg Cd per kilogram body weight per day (FAO/WHO 1972). There is obvious concern for the health of the scallop population as well.

Studies in our laboratory indicated that adductor muscle dissected from intact animals which had been stored frozen could be contaminated with Cd from other tissues, especially the digestive gland since sections of the muscle in contact with the digestive gland, contained higher Cd levels than did other sections of the muscle. This was not observed in cases where the animals were dissected live. In this instance Cd was equally distributed throughout the muscle.

We initiated a detailed study by bringing back approximately 150 live scallops from Georges Bank. These animals were used for two studies: 1) the distribution of Cd in all soft tissues of 6 scallops (100 mm shell height) by measuring the Cd content in each piece, and 2) the relationship between scallop shell height and the content of Cd in the digestive gland and the adductor muscle.

The digestive gland contained well over 90% of the total amount of Cd present in the soft tissues of the animal (Table 3). Dissection of scallop tissues was considerably more difficult than, say, lobster, since many of the tissues were fused to each other. Generally, a small amount of the digestive gland remained on the proximal end of the gonad and even after careful dissection this portion of the gonad contained a slightly higher concentration of Cd than the distal portion. The adductor muscle contained less than 1% of the total amount of Cd present in the soft parts of the animal. On a wet weight basis the average Cd concentrations were 0.121 ± 0.033 mg/kg in the adductor muscle and 98.3 ± 9.32 mg/kg in the digestive gland, an approximately 800 fold difference. Given the the well-known autolytic potential of digestive tissue, it is not surprising that post-mortem storage could lead to contamination of the adjacent adductor muscle with Cd.

Both the level of Cd (mg/kg wet wt) in the digestive gland and the Cd tissue burden (µg) in the digestive gland were positively correlated with the size of the animal (shell height in mm).

The resulting linear equations were:

$$[Cd]\ \text{digestive gland} = -6.183 + 1.382\ (\text{height}) \qquad r^2 = 0.665$$

and

$$Cd\ \text{total} = -629.1 + 10.70\ (\text{height}) \qquad r^2 = 0.819$$

Although a number of other biological parameters (e.g. sex, tissue weights and age) have been measured, their relationships to the amount or concentration of Cd in the digestive gland have not been studied yet. Analyses of Cd in the adductor muscles from the stratified sample are continuing.

Table 3. Cd in soft parts of sea scallop (100 mm shell height, N = 6).

Tissue	Amount of Cd in tissue (µg)	% of total Cd in soft tissues
Adductor muscle (+ tough muscle)	1.93 ± 0.54[a]	0.56
Adductor muscle (- tough muscle)	1.81 ± 0.53[b]	0.53
Mantle	3.97 ± 1.32	1.19
Gonad	1.41 ± 0.52	0.42
Gill	4.28 ± 1.47	1.28
Kidney	14.78 ± 6.49	4.39
Digestive gland	313.75 ± 35.31	92.1
Foot	0.241 ± 0.239	0.068
Resilium	none detected	
Soft tissues	340.4 ± 33.2	

a - equivalent to 0.121 ± 0.033 mg Cd/kg adductor muscle (wet wt)
b - tough muscle contained 0.115 ± 0.027 mg Cd/kg (wet wt)

The relationship between the Cd burden in the digestive gland and shell height and the observation that the digestive gland contains over 90% of the total amount of Cd in the soft tissues of the scallop suggest that this tissue is the proper tissue to study in developing temporal and spatial trends of Cd in scallop populations.

HEAVY METAL INTERACTION IN JUVENILE LOBSTER

Various levels of Cd were fed to juvenile lobsters in either casein-based (0 and 2.5 mg added Cd/kg diet) or crab-based (0, 2.5, 5.0, 10, 20, and 45 mg added Cd/kg diet) diets. (Table 4). The crab-based diets contained an additional 5 mg Cd/kg contributed by the crab meat. One additional group was fed with the 25 mg added Cd/kg crab-based diet without the usual addition of ascorbate (Vitamin C). Zn, Cu, and Ag levels were controlled in each diet but differed with the dietary protein source (Chou et al. 1983). Growth and survival were monitored during the course of the feeding. After 17 wk of feeding the surviving animals were starved for 48 h, weighed, digestive gland and tail muscle were removed from 3-5 animals, and pools of each tissue type prepared. Levels of Cd, Zn, Cu, and Ag were measured in each tissue pool by atomic absorption spectrophotometry.

Table 4. Cd, Zn, Cu, and Ag levels (mg/kg) in diets fed to juvenile lobsters. Diet composition is described in Chou et al. (1981, 1983).

Diet	Total Cd	Added Cd	Cu	Zn	Ag	
Casein	0	0	16.7	0	0	
Casein	2.5	2.5	16.7	60	0	
Crab	7.5	2.5	76.7	155	4.1	
Crab	10.0	5.0	76.7	155	4.1	
Crab	15.0	10.0	76.7	155	4.1	
Crab	25.0	20.0	76.7	155	4.1	
Crab	25.0	20.0	76.7	155	4.1	(no ascorbate)
Crab	45.0	40.0	76.7	155	4.1	

RESULTS

Survival within the various dietary groups averaged about 80%. A multipe range test indicated that there was no effect of diet on survival. Weight gain was optimal with the crab-based diets, containing 15 or 25 mg added Cd/kg. Overall, crab-based diets resulted in some greater weight gain compared to the casein-based diets (multiple range test).

Cd was taken up by both the digestive gland and the tail muscle. The concentration of Cd in the digestive gland from the crab-based diet was linear over the range of the dietary Cd levels and can be described by the equation:

$$\text{Mean [Cd] digestive gland} = 57.13 + 12.46\ [\text{Cd}]\ \text{diet}$$

with a coefficient of determination of 0.999. The concentration of Cd in the digestive gland of lobsters fed the casein-based diet was not as great as that of the crab-based diet due to the lower amount of Cd in the former diet.

The Cd concentration in the tail muscle from lobsters fed the crab-based diets was not linearly related to dietary Cd levels, the response being described by the equation:

$$\text{Mean [Cd] tail muscle} = 0.055 + 0.074 \log\ [\text{Cd}]\ \text{diet}$$

with a coefficient of determination of 0.983. Cd levels in the tails muscle were very much lower than corresponding digestive gland levels and it was not possible to distinguish a difference between the crab- and casein-based diets in the tail muscle Cd levels.

The lack of ascorbate in the crab-based diet with 25 mg added Cd/kg did not affect the level of Cd found in the tail muscle but significantly elevated the digestive gland Cd level.

As expected, due to the higher concentration of Zn in the crab-based diet, the level of Zn in the digestive gland was much higher in animals fed the crab-based diet than those fed the casein-based one. The addition of 60 mg Zn/kg to the casein-based diet resulted in a higher digestive gland level of Zn in those animals fed this diet than in those fed the unfortified diet. The effect of varying Cd levels in the crab-based diet upon the level of Zn in the digestive gland was complex and characterized by a large variance. The lack of ascorbate did not significantly affect the Zn level in the digestive gland. Muscle Zn levels were extremely consistent with only a small increase in muscle Zn levels (approximately 10%) being found in those animals fed the casein-based diet which had been fortified with 60 mg Zn/kg. Varying the amount of Cd in the crab-based diet did not significantly affect muscle Zn levels. The lack of ascorbate in the crab-based diet did not affect muscle Zn levels.

Cu was present naturally in the crab-based diet at a level 4.6 times that in the casein-based diet. The levels of Cu in the digestive glands from lobsters fed the crab-based diet ranged around 600 mg/kg (wet wt) while those in lobsters fed the casein-based diet were around 10 mg/kg, suggesting that Cu naturally present in the crab used to prepare the diet is more bioavailable than Cu added to the casein-based diet. No such difference was noted in the Cu levels in the tail muscles from the two groups; the tail muscles from the lobsters fed the crab-based diet contained slightly higher Cu levels than the tail muscles from animals fed the casein-based diets. The effect of varying the amount of Cd in the crab-based diets on Cu levels was complex in both tissues. The lack of ascorbate in the diet did not affect Cu levels in either tissue.

Ag was not detectable in the casein-based diet. The crab-based diet contained 4.10 mg Ag/kg (wet wt). Ag levels in the digestive glands of lobster fed the crab-based diet ranged from 8-15 mg/kg (wet wt) while the levels in the digestive glands of animals fed the two casein-based diets were 0.089 and 0.067 mg Ag/kg (wet wt) for the basal casein-based diet and the casein-based diet fortified with 2.5 mg Cd/kg, respectively. The tail muscles of lobsters fed crab-based diets contained higher levels of Ag than those from lobsters fed the casein-based diets but the magnitude of the difference was not as great as that observed with the digestive glands, the difference being approximately fourfold. The response of Ag levels in both the tail muscle and the digestive gland to varying amounts of Cd in the crab-based diet was complex. There was no observed effect on Ag levels in either tissue with the diet deficient in ascorbate.

As mentioned above, with the exception of tail muscle Zn levels, the effect of varying the amount of dietary Cd in the crab-based diets on tissue levels of Zn, Cu and Ag was complex. In general, as the amount of added Cd increased from 2.5-5.0 mg/kg of diet, the levels of these metals in the tissues increased then decreased as the added Cd level was raised to 10 mg/kg. As the added Cd level was further raised to 45 mg/kg the digestive gland Zn level increased to a level comparable to that found with the addition of 2.5 mg Cd/kg. Between 10 and 20 mg added Cd/kg diet digestive gland Cu levels increased somewhat with no noticeable difference occurring upon addition of 45 mg Cd/kg. Tail muscle Cu levels increased gradually (7-9 mg Cu/kg wet wt) between 10 and 45 mg Cd/kg diet. Digestive gland Ag levels decreased by about 20% as the added Cd level increased from 10-45 mg/kg while tail muscle Ag levels increased approximately 40%.

The two diets also had an effect on the relative size of the digestive glands in the lobsters. Digestive glands weights did not vary significantly within the two diets. Lobsters fed casein-based diets had a larger mean digestive gland weight/total weight ratio (0.0633±0.0052) than those fed the crab-based diet (0.0539±0.00667). In studies of wild adult lobsters a ratio of 0.0522± 0.0012 has been reported by Stewart et al. (1967).

STUDIES ON THE NATURE OF HEAVY METALS IN LOBSTER

Many heavy metals, e.g. Cd and mercury (Hg), occur in tissues in a variety of chemical forms, especially if the tissue has been stored or processed into food (Uthe et al. 1982b). Earlier, we applied differential pulse polarography to the determination of the amount of "free" Cd in shellfish tissue (Chou et al. 1978). In a study of the bioavailability of Cd in canned lobster digestive gland to rats we found that the amount of Cd taken up by the rat was related to the measured concentration of "free" Cd in the diet rather than to total Cd concentration (Utne and Chou 1980). Differentiation between protein-bound and unbound metal ions in tissue is generally carried out by using size-exclusion (molecular sieve) chromatography, a fairly time-consuming procedure since large columns are used and each column fraction must be analyzed. We have developed a rapid size-exclusion chromatography-flame atomic absorption spectrophotemetric method for distinguishing and measuring high and low molecular weight forms of Cd, Cu and Zn using short chromatographic columns with relatively high flow rates (from the spectrophotometric aspirator vacuum). One determination takes 10-15 min when this apparatus is used.

Historically, elution buffers for size exclusion chromatography have had relatively low ionic strengths. In our first attempts to quantitatively measure bound and "free" Cd substantial losses of Cd ions added prior to chromatography occurred with use of low ionic strength buffers (0.05 M Tris) and Sephadex G-50 or G-75 (a dextran material). Use of buffers of higher ionic strength (1-2 M NH_4OAc) resulted in quantitative elution of Cd ion from the column at the expected time. No problems were encountered with Zn ions. Severe problems were encountered with Cu ions, and it was necessary to use Cu binding agents such as EDTA or glycyl-glycine to remove Cu from the Sephadex column. Obviously, use of such reagents could be expected to lead to removal of metal ions from certain high molecular weight complexes. We have investigated the performances of other size exclusion materials and the results of these investigations are shown in Table 5. Of the four materials tested the best performances were found with Sephadex and Fracto-Gel. Elution of Cd, Zn and Cu ions was quantitative, using 1M ammonium acetate as an elution buffer. The technique has

Table 5. Elution of various metal ions by 1M NH_4OAc from different types of size exclusion-chromatographic materials. (S - quantitative elution of metal ions off column. NS - not satisfactory, some fraction of metal ions retained by column).

	Bio-Beads P-6	Sephadex G-25	Controlled Pore Glass CPG-40	Fracto-Gel HW-40
Cd	S	S	NS	S
Cu	NS[a]	S	NS	S
Zn	S	S	NS	S

[a] 2M NH_4OAc

been applied to analysis of Cd in lobster digestive gland extracts stored for some time without protease inhibitor to generate "free" Cd, and the chromatographic result obtained for "free" Cd compared with that determined by polarography (Table 6). It can be seen that the two methods gave equivalent results.

Table 6. Comparison of polarographic and chromatographic "free" (divalent) Cd results from extracts of lobster digestive glands. "Free" Cd values are equated to extract from 1 g digestive gland.

Sample No	Total Cd in digestive gland (µg/g wet wt)	Cd in extract from Tissue %	"Free" Cd		"Free" Cd in extract %
			Polarography µg	Size-exclusion chromatography µg	
JHW 54	583	90.3	102	105 (G-50) 110 (G-100)	20 21
M282	8.54	92.5	6.96	7.27 (G-50) 7.27 (G-100)	92 92

ORGANOCHLORINE CONTAMINANTS IN ATLANTIC COD (GADUS MORHUA)

Time Trend Study, Gulf of St. Lawrence

Analysis of the fifth year cod sample in the study of temporal trends of three representative and persistent organochlorine contaminants, polychlorinated biphenyls (PCB), hexachlorobenzene (HCB), and alpha-hexachlorocyclohexane (alpha-HCH) in cod livers of a length-stratified Gulf of St. Lawrence study (fork length 32-65 cm, age 3-11) has been completed. Linear regression of organ burden of PCB against fork length (unculled data) showed a positive correlation. Overall, no significant change has occurred in PCB levels in these fish since 1977 (analysis of covariance) despite the strict controls placed on the manufacture, use and disposal of PCB in recent years. This finding should be considered as preliminary until the data have been corrected for possible outliers and subjected to a complete statistical analysis (e.g. Scott et al. 1983).

Geographical Trend Study, Western Atlantic

Elsewhere, the geographical distribution study of organochlorine compounds in cod from Browns and Baccaro Banks, Grand Banks and Flemish Cap showed lower levels of PCB and p,p'-DDE, in the order of location as listed above (See Table 7). Levels of PCB in Browns Bank cod liver pooled samples (entire livers pooled) ranged from 0.62-1.30 mg/kg wet wt while the Flemish Cap values ranged from 0.32-0.50 mg/kg; p,p'-DDE in Browns and Baccaro Banks cod livers ranged from 0.16-0.30 mg/kg wet wt whereas Flemish Cap levels ranged from 0.10-0.14 mg/kg (in all cases the highest levels being present in the largest fish). HCB levels in Grand Banks and Flemish Cap livers were higher than Browns-Baccaro Banks livers. This may be an artifact due to the presence of interferences in the livers from Grand Banks and Flemish Cap. This study has been plagued with sampling problems. Catches only rarely yielded enough fish to fill each length interval specified by the sampling protocol. Also the Flemish Cap data are difficult to interpret due to an unusual PCB pattern which may indicate the presence of a different suite of contaminants. This should be investigated further.

CHLORODIBENZODIOXINS AND CHLORODIBENZOFURANS IN LOBSTER

With the cooperation of the Ontario Ministry of the Environment, which performed the chemical analysis of the tissue, we have completed a small investigation to determine if chlorodibenzodioxins and chlorodibenzofurans are present in the digestive gland of lobsters captured in the Miramichi River estuary and in the Bay of Chaleur, off Limestone Point. Two pooled samples of digestive glands from each of these areas were shipped frozen to Toronto along with an appropriate blank. The samples were analyzed by the usual methodology employed by the laboratory. The results are given in Table 8.

Currently, there is no tolerance for chlorodibenzofurans in food products. There is a tolerance of 20 ng/kg for 2,3,7,8-tetrachlorodibenzodioxin but none for total chlorodibenzodioxins.

These results suggest that there is no widespread occurrence of chlorodibenzodioxins at a level of concern to health officials. Levels of chlorodibenzofurans are higher and while no tolerance levels for these compounds in foodstuffs have been set as yet the situation may require further investigation.

INTERCOMPARATIVE STUDY OF THE DETERMINATION OF CHLOROBIPHENYLS

Under the auspices of the International Council for the Exploration of the Sea (ICES) an intercomparative study of the determination of chlorobiphenyls was carried out using herring oil. Each laboratory received a portion of the oil and another portion of the oil which had been spiked with undisclosed amounts of four chlorobiphenyls. Analysts were also supplied with small amounts of each of these chlorobiphenyls, an amount of Florisil and a standard procedure for cleaning up the oil utilizing Florisil. Analysts were asked to identify each of the supplied chlorobiphenyls, measure the concentration of each in both oils and measure all other organochlorine residues for which they had the capability, using both the supplied (ICES) and their own procedures.

Table 7. Levels of organochlorines in pooled cod livers from fish captured in various locations in the western Atlantic.

Pool number and length (cm)	PCB, mg/kg wet wt	HCB, mg/kg wet wt	p,p'-DDE, mg/kg wet wt	Number of fish in pool
		Grand Banks		
GB-15-20	0.29	0.014	0.06	4
GB-25-30	0.35	0.025	0.08	4
GB-35-40	0.48	0.053	0.18	8
GB-45-50	0.45	0.067	0.13	8
GB-55-60	0.64	0.034	0.19	7
GB-65-70	0.85	0.058	0.13	5
GB-75-80	0.85	0.067	0.17	5
		Flemish Cap		
FC-25-30	0.45	0.031	0.12	4
FC-35-40	0.41	0.031	0.10	8
FC-45-50	0.44	0.039	0.10	8
FC-55-60	0.32	0.030	0.07	1
FC-65-70	0.50	0.039	0.14	2
FC-70-75	0.38	0.041	0.12	3
		Browns and Bacarro Banks		
BB-35-40	0.62	0.011	0.19	8
BB-45-50	1.00	0.018	0.30	6
BB-55-60	1.30	0.021	0.16	2
BB-75-80	1.23	0.027	0.29	2
BaB-23	1.27	0.008	0.26	1
BaB-35-40	0.90	0.016	0.16	2
BaB-45-50	0.73	0.018	0.23	3

Table 8. Levels of chlorodibenzodioxins and chlorodibenzofurans in lobster digestive gland (ng/kg wet wt). Detection limit is 1-5 ng/kg, depending on degree of chlorine substitution.

Sample	Chlorodibenzodioxins		Chlorodibenzofurans	
	No. Cl	ng/kg	No. Cl	ng/kg
Limestone A (4 animals)	4,5,6[a]	nd[b]	4 (10)[c]	160
	7	3	5 (9)	95
	8	8	6,7,8	nd
Limestone B (4 animals)	4,5,6	nd	4 (10)	170
	7	4	5 (8)	110
	8	2	6,7,8	nd
Miramichi A (8 animals)	4,5,6	nd	4 (9)	19
	7	26	5 (5)	81
	8	11	6,7,8	nd
Miramichi B (8 animals)	4,5,6	nd	4 (8)	270
	7	1	5 (8)	140
	8	tr[d]	6,7,8	nd

[a]number of chlorines in the molecule. number of isomers shown in brackets
[b]not detected
[c]largest peak was not 2,3,7,8 isomer
[d]trace, at detection limit

Table 9. Mean concentration (ng/g) of chlorobiphenyls in unspiked herring oil analyzed by participants using their own standards. Only chlorobiphenyls which at least three laboratories reported on by at least three laboratories are considered here. Results in lower part of table refer to supplied chlorobiphenyls as reported by all participants.

Chlorobiphenyl	IUPAC number	ICES methodology $\bar{X}\pm$S.D.(C.V.)	N	Laboratory methodology $\bar{X}\pm$S.D.(C.V.)	N
	28	51.3 ± 6.8 (13)	4	65.6±24.4 (37)	5
	44	23.9 ± 6.8 (27)	4	37.8±14.9 (39)	5
	49	20.2 ± 8.1 (40)	4	31.6±22.4 (71)	4
	52	51.3 ± 9.0 (18)	5	61.0±13.6 (22)	5
	70	38.0 ± 8.3 (22)	4	43.0±12.9 (30)	6
	87	28.0 ±22.7 (81)	3	-	-
	95	85.5 ±41.8 (49)	5	68.8±15.6 (23)	4
	97	14.9 ± 6.8 (46)	4	22.3±15.2 (68)	5
	101	52.8 ±12.4 (23)	8	63.0±17.1 (27)	8
	107	44.2 ±25.9 (59)	3	24.7± 9.1 (37)	3
	110	39.8 ±13.0 (33)	4	42.1± 3.8 (9)	4
	128	11.0 ± 7.3 (66)	8	16.9±16.5 (98)	8
	138	57.4 ±20.1 (35)	11	72.3±19.0 (26)	10
	151	28.6 ±20.5 (72)	3	24.2±17.0 (70)	3
	153	82.6 ±32.9 (40)	7	87.1±39.5 (45)	8
	170	25.1 ±36.0 (143)	4	7.6± 1.6 (21)	4
	180	23.2 ±13.4 (58)	3	32.3±21.9 (68)	6
	187	14.6 ± 5.1 (35)	3	16.1± 3.3 (20)	4
	194	3.0 ± 2.0 (67)	3	2.8± 1.6 (57)	3
	201	3.4 ± 1.4 (41)	3	3.3± 1.1 (33)	3
	PCB	1.05± 0.36 (34)	21		
	52(CB-1)	74.1±52.9[a] (71)	29	72.8±51.0[a] (70)	28
	153(CB-3)	79.9±44.7 (56)	36	89.7±36.2 (40)	30
	101(CB-4)	21.6±109[a] (90)	38	140.2±113.6[a] (81)	31

[a]Mean estimated by Cochran's approximation (Snedecor and Cochran 1967) to be significantly higher than corresponding mean in upper part of table (5% level).

[b]analyzed as Aroclor 1254 or equivalent formulation (µg/g).

Only 17 of the 29 participants (excluding ourselves) supplied identification information on the four chlorobiphenyls (IUPAC numbers and chemical structures). An additional four participants identified only the number of chlorines present in each unknown chlorobiphenyl. None of these participants had difficulty identifying three of the chlorobiphenyls, namely, 2,2',5,5'-tetrachlorobiphenyl (CB-1), 2,2',4,5,5'-pentachlorobiphenyl (CB-4) and 2,2',4,4',5,5'-hexachlorobiphenyl (CB-3). The fourth one, 2,2',3,4,5-pentachlorobiphenyl (CB-2), was identified correctly by only two participants. Participants had corrected their results for recovery. In spite of this, the overall recovery of the added amount of CB-1 was less than 100%. The recoveries of CB-3 and CB-4 were satisfactory. Due to problems encountered in the identification of CB-2, the quantitative results for it were not analyzed. By using all participants' results for the other three chlorobiphenyls, it was determined that the majority of the overall variance in the results was due to systematic rather than random error (Youden and Steiner 1975). Analysis of the data submitted by those participants who had had experience in the measurement of chlorobiphenyls prior to this exercise showed that interlaboratory or systematic variance was lower but still a major contributor. The use of a standard method of clean-up (Florisil) did not result in substantial improvement in the results although the mean chlorobiphenyl values reported by the participants were significantly lower for the standard method compared with the participants' mean results determined by their own methodologies.

In order to recommend that analysts change from measurement of PCB to measuring individual chlorobiphenyls, the interlaboratory variance associated with measurement of each of the recommended chlorobiphenyls should be less than that associated with the measurement of PCB as currently

practiced. Table 9 lists the coefficients of variation for all chlorobiphenyls for which at least three of the participants submitted data. Since each of these participants had submitted data on chlorobiphenyls in addition to the four supplied ones they were judged to be "experienced" in the measurement of chlorobiphenyls. Participants were also requested to measure the amount of PCB present in the unspiked oil, using Aroclor 1254 or an equivalent formulation as a reference standard. The interlaboratory coefficient of variation associated with this latter measurement was 34%. In an earlier study we found an interlaboratory coefficient of variation of 31% (with the major contributor to the variance identified as systematic error) associated with the measurement of PCB in an unspiked fish oil (Uthe and Musial 1982). In the present study the only chlorobiphenyls which met the 34% criterion were IUPAC Nos. 52, 70, 101, 110 and 187. The coefficients of variation associated with IUPAC Nos. 28, 44, 95 and 201 were of the same magnitude while IUPAC Nos. 49, 97, 107, 128, 151, 153, 180 and 194 had coefficients of variation which were all greater than the criterion. Due to the small number of laboratories submitting data on chlorobiphenyls other than those supplied, it is difficult to interpret the variances further. This suggests that experienced analysts could begin interlaboratory studies on levels of five chlorobiphenyls. The coefficient of variation of the results submitted by all participants for each supplied chlorobiphenyl was significantly greater than that for the results from the "experienced" participants and in all cases except for CB-3 determined with the ICES methodology significantly greater (Sokal and Rohlf 1965) than the 34% criterion. This suggests that inexperienced analysts are having difficulty in quantitative chlorobiphenyl analysis.

STABILITY OF PCB IN FISH OIL

Ampules of herring oil, both unspiked and spiked with 1 mg Aroclor 1254/kg (about 1.3 and 2.3 mg/kg respectively) and stabilized with an antioxidant (vitamin E) were stored at -20°C and analyzed for PCB nine times over a 4-yr period (Musial and Uthe 1983). Over time the amounts of PCB measured in the two oils decreased by about 15%. However, the difference in PCB levels between the two oils remained constant, and after 2-yr was still equal to the original amount of Aroclor 1254 added.

Two different batches of Florisil were used during the 4-yr period for cleaning up the oil prior to gas chromatographic analysis. Inspection of the individual chromatograms showed that the measured PCB concentration in both oils was related to the batch of Florisil used for cleanup. The apparent decrease in the measured PCB concentration in both oils was probably due to batch-to-batch variability in the absorptive capacity of Florisil for non-PCB components which co-eluted with PCB during quantitation by gas chromatography.

REFERENCES

Chou, C.L., J.F. Uthe, J.D. Castell, and J.C. Kean. 1983. Heavy metal interactions in juvenile American lobster (Homarus americanus). Poster session for the Tenth Aquatic Toxicity Workshop, Halifax, N.S. November 1983.

Chou, C.L., J.D. Castell, J.F. Uthe, and J.C. Kean. 1981. Dietary copper requirement and possible role of silver in lobster (Homarus americanus). Poster session for the Proceedings of the 2nd International Conference of Aquaculture Nutrition, October 1981.

Chou, C.L., J.F. Uthe, and E.G. Zook. 1978. Polarographic studies on the nature of cadmium in scallop, oyster and lobster. J. Fish. Res. Bd. Can. 35: 409-413.

FAO/WHO (Food and Agriculture Organization of the World Health Organization). 1972. Evaluation of certain food additives and the contaminants mercury, lead and cadmium. World Health Organization, Tech. Rep. Ser. No. 505.

Musial, C.J., and J.F. Uthe. 1983. A study of the stability of polychlorinated biphenyl in fish oil. International Council for the Exploration of the Sea, CM:1983/E:37. 4 p. mimeo.

Ray, S. 1980. Distribution of cadmium in marine biota in the vicinity of Belledune, In: Cadmium pollution of Belledune Harbour, New Brunswick, Canada. J.F. Uthe and V. Zitko (eds.) Can. Tech. Rep. Fish. Aquat. Sci. 963: p. 11-27.

Reid, R.N., J.E. O'Reilly, and V.S. Zdanowicz. 1982. Contaminants in New York Bight and Long Island Sound sediments and demersal species and contaminant effects on benthos, summer 1980. NOAA Technical Memorandum NMFS-F/NEC-16: 96 p.

Scott, P.P., J.F. Uthe, and C.L. Chou. 1983. Choice of variables in estimation of time trends in contaminant levels in fish. Paper presented to the 1983 Meeting of the ICES Working Group on Marine Pollution Baseline and Monitoring Studies in the North Atlantic, Copenhagen, Denmark. 25 p. mimeo.

Snedecor, G.W., and W.G. Cochran. 1967. Statistical methods. Sixth Edition. The Iowa State University Press, Ames. 593 p.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. The principles and practice of statistics in biological research. W.H. Freeman, San Francisco 776 p.

Stewart, J.E., J.W. Cornick, D.M. Foley, M.F. Li, and C.M. Bishop. 1967. Muscle weight relationship to serum proteins, hemocytes, and hepatopancreas in the lobster Homarus americanus. J. Fish. Res. Bd. Canada 24: 2339-2354.

Uthe, J.F., and C.L. Chou. 1980. Cadmium levels in selected organs of rats fed three dietary forms of cadmium. J. Environ. Sci. Health A15: 101-109.

Uthe, J.F., and V. Zitko. (eds.) 1980. Cadmium pollution of Belledune Harbour, New Brunswick. Can. Tech. Rep. Fish. Aquat. Sci. 963: v + 107 p.

Uthe, J.F., C.L. Chou, and D.G. Robinson. 1980. Cadmium in American lobster (Homarus americanus) from the area of Belledune Harbour, In: Cadmium pollution of Belledune Harbour, New Brunswick, Canada. J.F. Uthe and V. Zitko (eds.). Can. Tech. Rep. Fish. Aquat. Sci. 963. p. 65-76.

Uthe, J.F., and C.J. Musial. 1982. Report on the fourth intercalibration study of PCBs in biological material. ICES Cooperative Research Report No. 115. Int. Counc. Explor. Sea. Copenhagen, Denmark 53 p.

Uthe, J.F., C.L. Chou, D.G. Robinson, and R.L. Levaque Charron. 1982a. Cadmium contamination of Belledune Harbour, New Brunswick, Canada - Studies on American lobster (Homarus americanus) during 1981. Can. Tech. Rep. Fish. Aquat. Sci. 1060: iii + 10 p.

Uthe, J.F., H.C. Freeman, G.R. Sirota, and C.L. Chou. 1982b. Studies on the chemical nature of and bioavailability of arsenic, cadmium, and lead in selected marine fishery products, p. 105-114. In: Chemistry and Biochemistry of Marine Food Products. K.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (eds.), AVI Publishing. p. 109-113.

Uthe, J.F., C.L. Chou, D.G. Robinson, and R. Levaque Charron. 1983. Cadmium in American lobster. In: Proc. Ninth Aquatic Toxicity Workshop, November 1982. Can. Tech. Rep. Fish. Aquat. Sci. 1163. p. 194-197.

Youden, W.J., and E.H. Steiner. 1975. Statistical manual of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Washington, D.C. 89 p.

CONTAMINANTS AND HORMONE METABOLISM

H.C. Freeman
G.B. Sangalang
G.R. Sirota

The objective is to develop and apply methods for the detection of the sub lethal effects of trace quantities of chemical contaminants on fish. Fish are sensitive to low levels of some toxins and therefore can be used to detect early pollution so adjustments can be made before problems become acute and irreversible.

ISOLATION AND IDENTIFICATION OF STEROID HORMONES IN LOBSTER

Steroid hormones are known to regulate the physiology of fish and are, therefore, critical to their life history. By determining changes in steroid hormone metabolism caused by pollutants, it is possible to predict how these compounds may affect fish physiology.

As part of our study to determine the sub lethal effects of pollutants on the American lobster (Homarus americanus), the principal steroid hormones in the blood of the lobster are being isolated, identified, and quantified. Recently, testosterone (T) was isolated and identified from lobster serum and testes (Burns et al. 1983a). Another steroid, 20-alpha-dihydroprogesterone, was also identified as a major metabolite in lobster testicular incubations in vitro (Burns et al. 1983b). Currently, a compound, tentatively identified as 20-alpha-dihydroprogesterone, has been isolated from lobster serum. Confirmation of its identity is in progress.

High performance liquid chromatography (HPLC) has become an important tool for steroid research. Methods have been developed where HPLC has been used efficiently in the purification, identification, and quantification of steroid hormones. HPLC has been used effectively in purifying plasma extracts of steroids for radioimmunoassay (RIA) analyses. This method when used for routine hormone isolation and analysis will result in considerable savings in both time and materials over the common thin layer chromatography procedures.

ACID RAIN STUDIES

In July 1982, 108 sexually maturing 2-yr-old Atlantic salmon were tagged and equal numbers of each sex were placed in cages in the Westfield and Medway Rivers until spawning time (Freeman 1982).

At transfer and then at 2- to 3-wk intervals until spawning time, each fish was removed, anaesthetized with MS222, weighed and approximately 1 mL of blood (heparinized) was taken. Blood plasma was stored at -40°C until analyzed for 11-ketotestosterone (11-KT) and testosterone (T) (Sangalang and Freeman 1977, 1978). Riverine pHs and temperatures were measured at each sampling time. At spawning time, the fish were anaesthetized, weighed, spawned, and sampled for blood. The mean diameter of the eggs was determined and the packed cell volume (spermatocrit) of the milt was determined, using standard microhematocrit procedures. The eggs from Medway and Westfield fish were fertilized with milt from fish from their same groups, incubated at the nearby Mersey Hatchery (Nova Scotia), and the mortality of the eggs to the hatching stage was determined.

In 1982 the pH of the Medway River waters was higher than that of the Westfield River with ranges of 5.4-6.2 and 5.1-5.3, respectively. However, the pH of the Westfield River water during the study period of August-November 1982 was considerably higher than in November 1981 when the pH was 4.7. The higher pH in 1982 was possibly due to the low rainfall during the fall of 1982 resulting in low water levels in the Westfield River and presumably contributing to the higher pH.

The mean body weight gains for Westfield River and Medway River salmon were 9.60% and 21.7% respectively, after 70 days of holding and 0.37% and 4.05%, respectively, before and after spawning (Freeman et al. 1983). The Westfield fish fed poorly and were not as active as the Medway fish. These results suggested that conditions in the Westfield River were not suitable for holding salmon. The mean volume of eggs from the Medway fish was 16% greater than that from the Westfield fish. Egg mortality to the eyed stage for the Westfield eggs was 90.9% compared to 59.3% for the Medway fish. Glebe et al. (1979) reported no significant correlation between egg size and survival suggesting that the high mortality of the Westfield salmon eggs is not associated with size. The mean packed cell volume of the milt from the Medway River fish (31.7 ± 8.4) was significantly different from that the Westfield River fish (40.8 ± 12.8) (F test).

In normal male Atlantic salmon, plasma levels of 11-KT and T become greatly elevated during the last few months of sexual maturation and peak at full sexual maturity (Idler et al. 1971; Sangalang and Freeman 1974; Stuart-Kregor et al. 1981; Hunt

et al. 1982; Billard et al. 1982). Plasma levels of these steroids in sexually mature wild male salmon captured in the Westfield river in the fall of 1981 were abnormally low (Freeman et al. 1983). It was thought that the cause was associated with the low pH (4.7) of the Westfield River (Freeman et al. 1983).

In 1982 the mean concentration of 11-KT in the plasma of both Medway and Westfield caged male salmon increased as the fish matured sexually (Fig. 1). The levels of this androgen peaked at or near functional sexual maturity. The peak mean levels of 11-KT for Medway River and Westfield River fish were 62.0 + 20.1 ng/mL and 40.3 + 25.9 ng/ml of plasma, respectively (Fig. 1). These hormone levels are within the range reported by Hunt et al. (1982) where 11-KT levels ranged from 25 to 70 ng/mL in the blood of sexually mature Atlantic salmon in Scotland also during the months of October and November. The mean plasma levels of 11-KT in male salmon from the Westfield and Medway Rivers and 22 wild ripe male salmon from several Nova Scotia rivers sampled by us on November 2-12, 1982, at the Coldbrook Hatchery, Coldbrook, N.S., were approximately the same (ca. 40 ng/mL); however, the mean concentration of 11-KT in the plasma of the male Westfield salmon had decreased sharply by November 23, 1982 (Fig. 1). This differed from the levels in the Medway River fish (Fig. 1) and from the data of Hunt et al. (1982). These results suggest that the metabolism or utilization of 11-KT was abnormal in the Westfield River salmon even when the pH was slightly over 5 and the river less acidic than it had been in 1981. In November 1981, when the Westfield River had a pH of 4.7, we found very little or only a trace of 11-KT in the blood of wild, mature Atlantic salmon captured in this river.

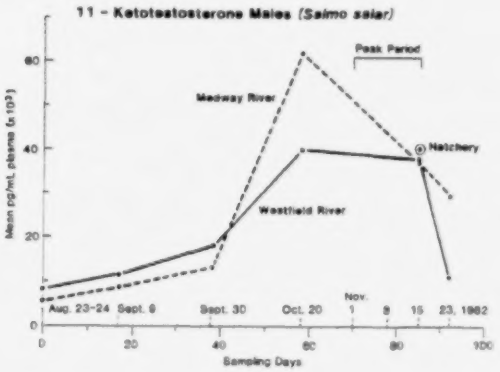

Fig 1. Blood 11-ketotestosterone levels of male Atlantic salmon (Salmo salar) held in the Medway and Westfied Rivers from August-November 1982.

Plasma T levels in sexually maturing Atlantic salmon held in the Medway River increased as the fish matured and peaked (20.8 + 10.9 ng/mL) at or near sexual maturity. The plasma T levels were also within the normal range for the various stages of sexual maturation. In contrast, the mean level of plasma T of the Westfield River fish (19.6 + 10.3 ng/mL) did not correlate with the approach of functional sexual maturity as expected. There was no decline in plasma T after sexual maturity (Fig. 2) as normally occurs. A similar elevated plasma level of T after spawning and at the onset of testicular regression was observed in brook trout that were held in water containing sublethal levels of Cd (Sangalang and Freeman 1974). The results of the present study suggest that the elevated T levels in the male Westfield Atlantic salmon may be a manifestation of the sublethal toxicity of some pollutants associated with the low pH of the river water.

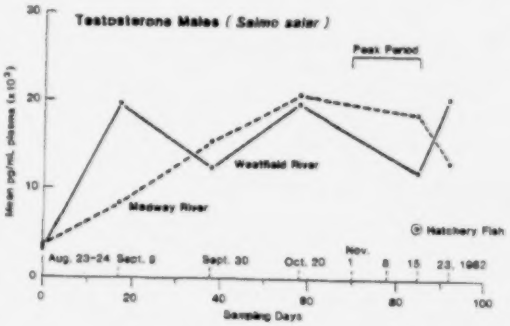

Fig 2. Blood testosterone levels of male Atlantic salmon (Salmo salar) held in the Medway and Westfield Rivers from August-November 1982.

Salmon were also held in the Medway and Westfield Rivers in 1983 until sexual maturity. In 1983 the pH of the Westfield River during the summer and early fall, due to low rainfall, was also higher than in 1981. From preliminary results, the Atlantic salmon held in the Westfield again gained less weight. The blood hormone analyses have not been completed for 1983.

Although the physiological effects of low pH waters on salmon are obscure at this time, the results of these comparative studies strongly suggest interference with sexual maturation and reproduction by low pH waters.

RADIOIMMUNOASSY (RIA) PROCEDURES

Radioimmunoassy (RIA) procedures were developed in our laboratory (Sangalang and Freeman 1977, 1978) and have been used routinely both for sexing immature fish and for determining blood hormone levels during sexual maturation. The techniques are valuable as multiple samplings are feasible without adversely affecting a significant number of animals. Less than 0.1 mL blood is required.

Over 2450 blood androgen levels were determined in 1983. The majority of these determinations were associated with the study of the effects of acidic water on maturation and reproduction in Atlantic salmon (Salmo salar). In cooperation with the Applied Fish Physiology and Salmon Genetics Research Section over 200 determinations of blood androgen levels were carried out. Plasma from lake trout (Salvelinus namaycush) was analyzed for the Canada Centre for Inland Waters.

During the past year RIA procedures have been developed for measuring levels of the estrogens, and estriol in fish plasma. At presently RIA procedures are being developed for determining levels of estriol-17-β in fish plasma.

ACKNOWLEDGEMENTS

The authors and the editor thank Ms. V. Clayton for typing, K. Deagle and F. Cunningham for art work, Ms. R. Garnett for editing and Dr. D.J. Scarratt for his constructive comments on the manuscript.

REFERENCES

Billard, R., A. Fostier, C. Weil, and B. Breton. 1982. Endocrine control of spermatogenesis in teleost fish. Can. J. Fish. Aquat. Sci. 39: 65-79.

Burns, G., G.B. Sangalang, H.C. Freeman, and M. McMenemy. 1983a. Isolation and identifiction of testosterone from the serum and testes of the American lobster (Homarus americanus). Gen. Comp. Endocrinol. (accepted)

Burns, G., G.B. Sangalang, H.C. Freeman, and M. McMenemy. 1983b. Bioconversion of steriods by the testes of the American lobster Homarus americanus) in vitro. Gen. Comp. Endocrinol. (accepted)

Freeman, H.C. 1982. The effects of an acidic river on blood sex hormone levels in sexually mature male Atlantic salmon (Salmo salar), p. 117-121. In: Dept. of Fisheries and Oceans, Fisheries Research Branch Workshop on Acid Rain, Project Reports, 1981-82. St. Andrews, N.B. March 2, 1982.

Freeman, H.C., G.B. Sangalang, G. Burns, and M. McMenemy. 1983. The blood hormone levels in sexually mature Atlantic salmon (Salmo salar) in the Westfield River pH (4.7) and Medway River (pH 5.6), Nova Scotia. The Science of the Total Environment. (accepted)

Glebe, B.D., T.D. Appy, and R.L. Saunders. 1979. Variation in Atlantic salmon (Salmo salar) reproduction traits and their implication in breeding programs. International Council for the Exploration of the Sea, C.M. 1979/M:23.

Hunt, S.M.V., T.H. Simpson, and R.R. Wright. 1982. Seasonal changes in the levels of 11-testosterone and testosterone in the serum of male salmon Salmo salar L., and their relationship to growth and maturation cycle. J. Fish. Biol. 20: 105-119.

Idler, D.R., D.A. Horne, and G.B. Sangalang. 1971. Identification and quantification of the major androgens in testicular and peripheral plasma of Atlantic salmon (Salmo salar) during sexual maturation. Gen. Comp. Endocrinol. 16: 257-267.

Sangalang, G.B., and H.C. Freeman. 1974. Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production in vivo in brook trout. Biol. Reprod. 11: 429-435.

Sangalang, G.B., and H.C. Freeman. 1977. A new sensitive and precise method for determining testosterone and 11-ketotestosterone in fish plasma by radioimmunoassay. Gen. Comp. Endocrinol. 32: 432-439.

Sangalang, G.B., and H.C. Freeman. 1978. A simple technique for determining the sex of fish by radioimmunoassay (RIA) using 11-ketotestosterone antiserum. Gen. Comp. Endocrinol. 36: 187-193.

Stuart-Kregor, P.A.C., J.P. Sumpter, and J.M. Dodd. 1981. The involvement of gonadotrophin and sex steroids in the control of reproduction in the parr and adults of Atlantic salmon Salmo salar L. J. Fish. Biol. 18: 59-72.